THE TOXIN OF BACILLUS WELCHII. II

THE MECHANISM OF INFECTION WITH B. WELCHII

PAUL H. DEKRUIF AND JESSE L. BOLLMAN

From the Hygienic Laboratory, University of Michigan, Ann Arbor.

It is generally accepted that the human body is highly resistant to infection by Bacillus welchii. Westenhoeffer,[1] for instance, claims that this organism is a pure saprophyte and is able to multiply in dead tissue only. It is the experience of surgeons that gas gangrene infections establish themselves only after severe trauma, which damage tissues badly and cut off the circulation from certain areas. This explains the first stage of the infection, but leaves its subsequent extension unaccounted for. It is possible that the analysis of the conditions of experimental infection in animals might throw light on this question.

Guinea-pigs are susceptible to infection with the majority of strains of B. welchii, but it is well known that comparatively large amounts of the culture have to be injected to produce infection. The opinion is generally held that this is due to the injection of the highly acid culture medium along with the organisms. The acid is present in a concentration sufficient to damage the tissue, thus preparing it for the growth of the organism. Hitschmann and Lindenthal[2] claim that the virulence of different strains of B. welchii varies with their ability to produce acid. Simonds[3] was unable to produce infection in rabbits when he used large masses of bacilli taken from the surface of agar bottle slant cultures. He ascribes this failure to the fact that no acid or metabolic products, as would have been the case with liquid cultures, were injected along with the organisms.

The discovery by Bull and Pritchett[4] of the production of a soluble toxin by B. welchii suggests another explanation of the mechanism of infection. It is possible that the tissue damage which paves the way for the experimental infection and results in its rapid extension may not be due to the acid at all, but rather to the injection, along with the organ-

Received for publication Oct. 10, 1917.

[1] Virchow's Archiv., 1902, 170, p. 517, quoted from Simond.[3]

[2] Sitzungsber. d. k. Akad. d. Wissensch., math.-naturwissensch. Kl., 3te Abt., 1899, 108, p. 67, quoted from Simonds.

[3] Studies in Bacillus welchii, etc., Monographs of the Rockefeller Institute for Medical Research, No. 5, 1915.

[4] Jour. Exper. Med., 1917, 26, p. 119.

ism, of small amounts of toxin. This toxin has a strong necrotic effect on muscular tissue. It is likely then, that it may play the rôle of an aggressin, not in the sense of Bail, but rather as an agent which establishes conditions suitable for the growth of the "necro-parasitic" bacillus. This property of the toxin might account not only for the establishment of experimental infections but for the extension of infections in the human body. It is our purpose to show that this is indeed the case and that acid plays a small if any part in the experimental infection.

THE VIRULENCE OF NEUTRALIZED CULTURES

The organism used in this test was B. welchii, Strain 617d. The culture medium was a 0.2% glucose veal broth, incubated 18 hours. Three tubes of the 18-hour culture which showed a heavy growth of typical organisms were pooled, and subsequently divided into 2 parts. Part "A" was neutralized with normal

TABLE 1
THE VIRULENCE OF NEUTRALIZED CULTURES

Exper.	Guinea-Pig		C.c. of Neutral Culture	C.c. of Acid Culture	Result
	Number	Weight			
A	1	300	1.5	...	+ 14 hours; gas, edema
	2	300	1.0	...	+ 14 hours; gas, edema
	3	300	0.5	...	+ 17 hours; gas, edema
	4	300	0.2	...	+ 16 hours; gas, edema
	5	300	0.1	...	+ 17 hours; gas, edema
	6	300	0.01	...	Nil
	7	300	0.005	...	Nil
B	1	300	...	0.5	+ 17 hours; gas, edema
	2	300	...	0.1	+ 24 hours; gas, edema
	3	300	...	0.01	Nil
	4	300	...	0.005	Nil

NaOH; 0.08 c.c. of the normal soda per c.c. was required to neutralize the culture to phenolphthalein. Part "B" remained unneutralized and served as the control. The hind-legs and the abdominal surfaces of the guinea-pigs used were shaved, this procedure facilitating the observation of the course of infection. The injections were made with a fine needle deep into the thigh muscles, and were carried out at once after the neutralization of Part "A."

The first evidence of infection is the swelling of the injected leg, which is invariably painful to palpation. The swelling, which is edematous, rapidly spreads into the groin and finally into the abdominal surface.

Palpation of the leg and inguinal region reveals crepitation. Although we have observed recovery of guinea-pigs after infections in which the edema had extended to the abdominal surface, the presence of gas invariably indicates a fatal infection. The rapidity of death depends to a great extent on the amount of culture material injected. It varies with this factor from 4-48 hours. The result of this experiment is recorded in Table 1.

This experiment indicates that the virulence of neutralized cultures differs little if any from those not neutralized. In the case of both

"A" and "B" 0.1 c.c. of culture resulted in fatal infection while 0.01 c.c. failed to do so. It was not thought necessary to introduce values between 0.1 and 0.01 c.c., as such differences would be negligible when slight variations in individual animals are taken into account. If the culture medium possesses some injurious property which allows the organism to gain a foothold, it must be ascribed to some other factor than the acidity.

INFECTION WITH SUPERNATANT FLUIDS OF CENTRIFUGATED CULTURES

In studying the effect of the medium in which B. welchii has grown on the virulence of the organism it would be desirable to separate the medium from the organisms. In all subsequent experiments the organisms used for infection were separated from the culture medium by centrifugation and the resulting bacillary sediment was washed and

TABLE 2

INFECTIVITY OF CULTURE SUPERNATANT FLUIDS AFTER CENTRIFUGATION AT 8,000 R. P. M.

Supernatant	Guinea-Pig		C.c. Injected Intramuscularly	Result
	Number	Weight		
I	1	250	1.0	+ 5 hours, 30 minutes; gas, edema*
	2	250	5.0	+ 3 hours, 45 minutes; gas, edema*
II	1	700	1.5	+ 22 hours; gas, edema*
	2	700	2.0	+ 21 hours; gas, edema*

* Necropsy typical. Numerous bacilli in exudate.

centrifuged twice with large volumes of sterile 0.85% NaCl solution. The centrifugation was in all instances carried out at 8,000 revolutions per minute. The organism sediment from the 2nd washing was suspended in an amount of NaCl solution equal to the orginal amount of the culture. The resulting suspension represented, c.c. for c.c., the number of organisms in the original culture. The sediments during the washing and when suspended before injection were carefully agitated in a bulb pipet to avoid the presence of clumps of organisms. The NaCl solution used for washing and final suspension was kept in ice in order to preserve as far as possible the viability of the organisms.

Simonds[3] states that 5 c.c. of a supernatant fluid from an egg-broth culture still contained, after 30 minutes of centrifugation, enough organisms to kill a guinea-pig in 21 hours. Bull and Pritchett[4] also note the fact that a few organisms left behind in the supernatant fluid after centrifugation were sufficient to cause infection. It would seem

necessary, therefore, to test the infectivity of supernatant fluids from cultures even after the violent centrifugation described above. If this were not done, the supposed effect of culture supernatants on washed organisms might be in reality due to the addition of organisms which failed to sediment.

This question was attacked in the following experiment. The supernatant fluid from an 18-hour 0.2% glucose broth culture of Strain 617d was neutralized to phenolphthalein with normal NaOH. The fluid had been obtained from the broth culture by 10 minutes of centrifugation at 8,000 r. p. m. The injections were made intramuscularly. Table 2 represents the results of 2 different experiments made with different supernatant fluids.

This experiment confirms the observations of the workers referred to. It indicates, moreover, that it would be unsafe to use a merely centrifugated culture supernatant. It is necessary to remove completely all organisms from the fluid before attempting to analyze the comparative importance of these 2 factors in infection. In all subsequent experiments the cultures were neutralized, centrifugated and filtered through Berkefeld N filters. The filtrates were then tested for sterility before use.

THE EFFECT OF CULTURE FILTRATES ON THE VIRULENCE OF WASHED B. WELCHII

Having determined the unimportance of acid as a factor in the initiation of infection, it was thought desirable to determine in a roughly quantitative manner the aggressive effect of the neutralized filtrates.

Three tubes of an 18-hour 0.2% glucose broth culture of Strain 617d were pooled, neutralized, centrifugated and filtered. The filtrate proved to be free from organisms. The sediment of bacilli was washed twice with large volumes of 0.85% NaCl solution and after the 2nd washing was suspended in a volume of NaCl solution equivalent to the original culture.

The suspension was now diluted 1:10, 100, 1,000, 10,000, and 100,000 in cold sterile NaCl solution. Each dilution was shaken carefully to insure perfect suspension. In the case of tests 7, 8, and 9, in both experiments A and B, 0.2, 0.3 and 0.5 c.c. of the original suspension were brought to 1 c.c. volume with sterile NaCl solution. In the rest of the tests 1 c.c. of the dilutions mentioned was injected.

Experiment A.—One c.c. of each dilution was mixed with 0.5 c.c. of neutral culture filtrate, drawn up into a syringe and injected intramuscularly.

Experiment B (Control).—One c.c. of each dilution was mixed with 0.5 c.c. of sterile 0.2% glucose broth and injected as in A.

Experiment C.—The culture filtrate was tested for toxicity.

The injections in the case of Expers. A and B were made in order from t' e higher to the lower dilutions. The guinea-pigs used weighed 250 gm.

Observation of Table 3 shows that the neutral filtrate is able to increase the virulence of the washed bacillus at least 10,000 times. It is unfortunate that higher dilutions in Exper. A were not made and

TABLE 3
THE EFFECT OF CULTURE FILTRATE ON WASHED ORGANISMS (617D)

Exper.	Number	C.c. of Washed Organisms	C.c. of Filtrate	C.c. of Broth	Result
A	1	0.00001	0.5	...	+ 19 hours; edema, gas
	2	0.0001	0.5	...	+ 14 hours; edema, gas
	3	0.001	0.5	...	+ 18 hours; edema, gas
	4	0.01	0.5	...	+ 15 hours; edema, gas
	5	0.05	0.5	...	+ 16 hours; edema, gas
	6	0.1	0.5	...	+ 14 hours; edema, gas
	7	0.2	0.5	...	+ 14 hours; edema, gas
	8	0.3	0.5	...	+ 13 hours; edema, gas
	9	0.5	0.5	...	+ 14 hours; edema, gas*
B	1	0.00001	...	0.5	0
	2	0.0001	...	0.5	0
	3	0.001	...	0.5	0
	4	0.01	...	0.5	0
	5	0.05	...	0.5	0
	6	0.1	...	0.5	+ 36 hours; edema, gas*
	7	0.2	...	0.5	+ 16 hours; edema, gas
	8	0.3	...	0.5	+ 16 hours; edema, gas
	9	0.5	...	0.5	+ 15 hours; edema, gas
C	1	...	0.5	...	0
	2	...	0.75	...	0
	3	...	1.0	...	0

* Necropsies typical.

tested, since it is possible that still fewer organisms, under the influence of the filtrate might have proven fatal. On the other hand, it is certain that the neutral filtrate possess enormous aggressive power. This experiment was repeated with equally good results.

It seemed desirable to test the effect of the filtrate on another strain of B. welchii. Strain 7, isolated in this laboratory from the stool of a normal individual was used in this experiment.

TABLE 4
THE EFFECT OF CULTURE FILTRATE ON WASHED ORGANISMS, STRAIN 7

Exper.	Number	C.c. of Washed Organisms	C.c. of Filtrate	C.c. of Broth	Result
A	1	0.0001	0.5	...	+ 19 hours; gas, edema
	2	0.001	0.5	...	+ 17 hours; gas, edema
	3	0.01	0.5	...	+ 16 hours; gas, edema
	4	0.1	0.5	...	+ 15 hours, 30 minutes; gas, edema
	5	1.0	0.5	...	+ 14 hours; gas, edema
B	1	0.05	...	0.5	0
	2	0.1	...	0.5	0
	3	0.2	...	0.5	0
	4	0.3	...	0.5	0
	5	0.5	...	0.5	+ 18 hours; gas, edema
	6	1.0	...	0.5	+ 21 hours; gas, edema

Injections were made intramuscularly. The guinea-pigs weighed 250 gm.

An 18-hour culture of this strain in 0.2% glucose broth was centrifugated and the suspension made in the usual manner. The organisms were washed twice and the final suspension was made up to original volume with 0.85% NaCl solution. The mixing of the filtrate and the broth (control) with the dilutions of the washed organisms was carried out in a manner exactly similar to that of the preceding experiment.

The aggressive action displayed by the filtrate in regard to Strain 617d is seen also to be present in the case of Strain 7. In the latter case the virulence of the organism is increased by at least 5,000 times. One ten thousandth of a c.c. of organism suspension kills when mixed with the neutral filtrate, while 0.5 c.c. is required to infect when mixed with plain glucose broth.

In the preceding experiments constant amounts of culture filtrates were used together with varying amounts of organisms. The next inquiry concerned the amount of the aggressive filtrate required to make a given amount of the washed organism suspension virulent. The amount of washed organisms chosen was 0.01 c.c., about one-tenth the amount usually required to infect when no aggressin is used.

TABLE 5

EFFECT OF VARYING AMOUNTS OF AGGRESSIVE FILTRATE ON THE VIRULENCE OF B. WELCHII (617D)

Exper.	Number	C.c. of Washed Organisms	C.c. of Filtrate	C.c. of Broth	Result
A	1	0.01	0.5	...	+ 18 hours; edema, gas
	2	0.01	0.3	...	+ 17 hours; edema, gas
	3	0.01	0.1	...	Mild infection, recovers
	4	0.01	0.075	...	0
	5	0.01	0.05	...	0
B	1	0,01	...	0.5	0
	2	0.01	...	0.3	0
	3	0.01	...	0.1	0
C	1	....	2.0	...	Severe edema, recovers
	2	....	1.5	...	Moderate edema
	3	....	1.0	...	0

The organism used was Strain 617d washed twice and suspended in sterile NaCl solution as usual. One c.c. of a 1:100 dilution of the organism suspension was mixed with varying amounts of the neutral filtrate. As controls, similar amounts of organism plus similarly varying amounts of 0.2% glucose broth were used. Controls of filtrate toxicity were also made.

The guinea-pigs used in this experiment weighed about 200 gm. The injections were made, as usual, intramuscularly. The result of the experiment is given in Table 5.

Reference to Experiment A in Table 5 shows that 0.3 c.c. is the smallest amount of supernatent fluid that will cause 0.01 c.c. to set up a fatal infection. Observation of Experiment C will indicate that the dose of filtrate having an aggressive action is far below the fatal toxic

dose. This does not mean, however, that the toxin and the aggressin are not one and the same thing. As we have remarked before, non-fatal doses of toxin will give rise to severe local necrotic effects.

THE SPECIFIC NATURE OF THE AGGRESSIN

We have shown that considerable concentrations of toxin can be obtained from 0.2% glucose broth without the addition of sterile muscle. It is entirely possible then that the aggressin may be identical with the toxin. The following experiment appears to show that this is in all probability the case.

TABLE 6

THE EFFECT OF HEAT AND OF ANTITOXIN ON THE AGGRESSIVE POWER OF NEUTRAL FILTRATES

Exper.	Number	C.c. of Washed Organisms	C.c. of 70 C. Filtrate	C.c. of Unheated Filtrate	C.c. of 1:50 Anti-toxin	C.c. 1:50 Horse Serum	Result
A	1	0.01	0.5	...	...	...	0
	2	0.001	0.5	...	...	...	0
B	1	0.01	...	0.5	...	...	+ 19 hours*
	2	0.001	...	0.5	...	...	+ 20 hours*
C	1	0.01	...	0.5	1.0	...	0
	2	0.001	...	0.5	1.0	...	0
D	1	0.01	...	0.5	...	1.0	+ 20 hours*
	2	0.001	...	0.5	...	1.0	+ 32 hours*

* Typical necropsy; gas, copious edema, many bacilli.

The organisms used were washed bacilli from an 18-hour glucose broth culture of Strain 617d. The neutral filtrate was secured from the supernatant fluid of this same culture.

Experiment A.—A small amount of the neutral filtrate was heated to 70 C. for 30 minutes and 0.5 c.c. were added to each of 2 tubes, containing 1 c.c. of 1:100 and 1:1,000 respectively of washed organisms. The mixtures were shaken thoroughly and injected at once intramuscularly.

Experiment B (Control).—There were added 0.5 c.c. of unheated filtrate to each of 2 tubes, containing the same amounts of bacilli as in Exper. A, mixed, and injected at once.

Experiment C.—In each of 2 tubes 0.5 c.c. of unheated filtrate was placed and to each of these were added 5 units of Welch antitoxin (1 c.c. of 1:50). The tubes were allowed to stand at room temperature for 30 minutes, then 1 c.c. of 1:100 and 1:1,000 washed organisms respectively were added to Tubes 1 and 2. The mixtures were shaken thoroughly and injected intramuscularly.

Experiment D (Control).—In Tubes 1 and 2, 0.5 c.c. of unheated filtrate was placed. Then 1 c.c. of 1:50 normal horse serum was added to each, and the mixtures allowed to stand for 30 minutes. To Tubes 1 and 2 were added 1 c.c. of 1:100 and 1:1,000 respectively of washed organisms. The mixtures were shaken and injected at once intramuscularly.

The guinea-pigs used in this experiment weighed 250 gm. The result is given in Table 6.

The result of this experiment is illuminating. Whereas in Experiment A Nos. 1 and 2, which received organisms treated with 70 C. filtrate, showed no sign of infection, the controls in Experiment B, Nos. 1 and 2, died promptly with edema and gas. Necropsy showed myriads of Welch bacilli at the site of inoculation and in the exudate on the abdominal surface. In the case of Experiment C, Nos. 1 and 2, in which the filtrate was treated with 5 units of antitoxin, no infection took place, while the controls in Experiment D, Nos. 1 and 2, died with typical infection just as did the controls, Nos. 1 and 2 of Experiment B.

The aggressin, therefore, is destroyed by heat of 70 C., and is neutralized by antitoxin, and it may be safely concluded that true toxin, present in small amounts in the neutral filtrates, is responsible for the aggressive action. It would be of interest to determine whether the toxin made in the usual manner and capable of killing in small doses, would exert a similar effect when injected together with washed organisms.

TABLE 7

THE AGGRESSIVE ACTION OF TOXIN FROM MUSCLE CULTURES

Exper.	Number	C.c. of Washed Organisms	C.c. of Filtrate	C.c. of Anti-toxin 1:250	C.c. of Normal Horse Serum 1:250	Result
A	1	0.01	0.1	1.0	...	0
	2	0.01	0.05	1.0	...	0
	3	0.01	0.01	1.0	...	0
B	1	0.01	0.1	...	1.0	+ 48 hours; gas, edema*
	2	0.01	0.05	...	1.0	Severe infection, recovered
	3	0.01	0.01	...	1.0	0

* Necropsy typical, many bacilli.

The toxin employed in this experiment was made as follows: 0.1% glucose veal broth was placed in a small Erlenmeyer flask and several pieces of freshly excised sterile rabbit muscle were added. The flask was then inoculated with about 1 c.c. of an 18-hour litmus milk culture of Strain 617d, exhausted, and after 18 hours' incubation filtered through a Berkefeld N. filter. The minimum lethal dose for guinea-pigs of 300 gm. weight was 0.3 c.c.

Experiment A.—In each of 3 tubes 0.1, 0.05, and 0.01 c.c. of the above toxin were placed and mixed with 1 c.c. of a 1:250 Welch antitoxin (1 unit). The mixtures stood at the temperature of the room for 30 minutes; then to each tube was added 0.01 c.c. of a washed suspension of 617d strain. The mixtures, after shaking, were immediately injected intramuscularly.

Experiment B (Control).—To 3 tubes containing the same amounts of toxin as in Experiment A, 1 c.c. of 1:250 normal horse serum was added. After 30 minutes, 0.01 c.c. of the washed organisms was added to each, and the mixture injected as in Experiment A. The guinea-pigs used in this experiment weighed 300 gm. The result of the experiment is given in Table 7.

As in the preceding experiment the toxin mixed with antitoxin has no aggressive power while similar amounts which have been treated with normal horse serum cause in the case of 0.1 c.c. fatal and of 0.5 c.c,. severe infections. Although the experiments present clinching evidence of the specific nature of the aggressin of neutral filtrates it might be well to determine whether the metabolitic products of organisms other than B. welchii might exert a nonspecific aggressive action.

We chose at random 2 different organisms, Proteus vulgaris and the cholera vibrio. These organisms were grown for 18 hours in 0.2% glucose broth, the growth, which was heavy, was centrifugated and the supernatant, after neutralization, was filtered as in the case of the aggressin of B. welchii. A neutral culture filtrate of B. welchii of known aggressive power was used as a control. The organisms were, as usual, washed cultures of the 617d strain.

TABLE 8

ATTEMPT AT NONSPECIFIC AGGRESSIVE ACTION

Exper.	Number	C.c. of Washed Organisms	C.c. of Welch Filtrate	C.c. of Cholera Filtrate	C.c. of Proteus Filtrate	Result
A	1	0.1	0.5	...	...	+ 17½ hours; gas, edema
	2	0.05	0.5	...	...	+ 18 hours; gas, edema
	3	0.01	0.5	...	...	+ 17 hours; gas, edema
	4	0.001	0.5	...	...	+ 23 hours; gas, edema
B	1	0.1	...	0.5	...	0
	2	0.05	...	0.5	...	Slight edema
	3	0.01	...	0.5	...	0
	4	0.001	...	0.5	...	0
C	1	0.1	...	...	0.5	Slight edema
	2	0.05	...	...	0.5	0
	3	0.01	...	...	0.5	0
	4	0.001	...	...	0.5	0

Experiment A.—There was added 0.5 c.c. of neutral filtrate to 1 c.c. of 1:10, 20, 100, and 1,000 c.c. of the washed organism. The mixtures were shaken thoroughly and injected at once.

Experiment B.—Like A, except that 0.5 c.c. of the cholera filtrate was used in each case.

Experiment C.—Like A, but proteus filtrate was used.

The suspensions of washed organisms in all 3 experiments came from the same pool. The injections were made intramuscularly into guinea-pigs which weighed 250 gm. The results are given in Table 8.

The neutral filtrates of 2 organisms other than B. welchii failed to exert an appreciable aggressive effect. The test is a rigid one, since the 0.1 c.c. dose of washed organisms is almost sufficient to kill when no neutral filtrate is added. And this brings up the question as to how washed organisms without the aid of the supernatant fluid are able to

cause infection. The minimal infecting dose for twice washed B. welchii, without the aggressin, varies between 0.1 and 1.0 c.c. It would seem apparent then, that the organism cannot be called a pure saprophyte, since beyond a certain dose it is able to infect without the aid of the aggressive filtrates. On the other hand, it is possible that some of the toxin is closely bound to the cell wall and in this way imparts a necrotic action to the bacilli themselves.

We have tried to approach this question by the testing of the comparative virulence of organisms washed twice and 5 times with large volumes of 0.85% NaCl solution. We found that the organisms after 5 washings were fully as virulent as those that had been washed twice. It is apparent then, that even the most thorough washing is unable to remove or destroy the ability of large quantities of organisms to infect. It is highly probable, though not proven, that we are dealing here with a preparatory necrotic action due to toxin closely bound to the bacterial cell bodies.

THE EFFECT OF NEUTRAL FILTRATES ON PHAGOCYTOSIS

The "aggressins" of Bail are supposed to operate through the negative chemotactic effect they exert on leukocytes. While we were quite certain that the major part of the aggressive effect of our neutralized cultures filtrates was due to a necrotic action, it was possible that these fluids might exert an injurious effect on the white blood cells. The idea suggested itself because of the frequently observed marked absence of phagocytes in the exudates of gas bacillus infections. We thought it best to approach this question by a study of infections produced by intraperitoneal inoculation, since it was likely that the mobilization of phagocytes would be more rapidly accomplished at this point than in muscular tissue.

Repeated experiments on this problem failed to disclose any decisive information. Occasionally large masses of phagocytes were found in the exudates of infections after intraperitoneal inoculation of broth cultures. At other times but few phagocytes appeared, the peritoneal fluid swarmed with rapidly multiplying organisms, and the animal died in a few hours. The use of the washed organisms and filtrates rendered equally indecisive results. Comparative tests were made on the effects of acid and neutral culture supernatant on the phagocytosis of B. welchii after intraperitoneal inoculation. The acid of the cultures did not seem to check phagocytosis, nor did the neutralized filtrate, as

compared with plain broth have any effect. It must be concluded that by far the most important aggressive activity of the toxin resides in its necrotic effect.

DISCUSSION

The foregoing experiments throw light on the mechanism of infections by B. welchii in animals and by inference on the method of extension of the infection in the human body. In the latter case the primary conditions for infection are established by the tissue damage resulting from the wound. The spores introduced at this time are able to germinate, and having passed into the vegetative stage, find in the injured muscular tissue an admirable medium for the production of the specific toxic substance. This substance, with its diffusibility aided by the outpouring of edema fluid that invariably accompanies infection, is able rapidly to necrose further tissue and so furnish new medium for the growth of the organisms. Finally an area of necrotic tissue sufficient to furnish medium for the growth of an enormous number of organisms is produced. These then are able to produce enough toxin to bring about toxemic death.

We have repeatedly tried to produce active immunity in rabbits and guinea-pigs by the injection of killed washed organisms. Such attempts have invariably failed, as have those to prevent infection by the use of a bacteriolytic serum. It seems to be very difficult to bring about any degree of opsonic or lytic immunity against B. welchii.

But in the light of the data brought forward in the preceding pages, the outlook for the prevention and treatment of gas gangrene is very bright. By the use of the specific antitoxin, which has been obtained in high potency by Bull, the all important aggressive factor is capable of neutralization. The only possibility standing in the way of remarkable results even in treatment is that of a comparatively greater affinity of the toxin for muscle than for antitoxin.

The resistance of the human body to B. welchii is very high and the primary invasion is accomplished by spores. It should be possible then, by proper methods of infiltration, to prevent infections from starting. And experiments just published by Bull,[5] indicate that this explanation of the method of infection is probably the correct one, since this investigator has been able in guinea-pigs to cure infections by antitoxin even after these had made great headway.

[5] Jour. Exper. Med., 1917, 26, p. 603.

SUMMARY AND CONCLUSIONS

The acidity of cultures of B. welchii is not the prime cause of their ability to produce experimental infections. Neutralized cultures produce fatal infections in guinea-pigs in practically the same amounts as do acid cultures.

Bacilli, removed from broth cultures by centrifugation and subsequently washed with large volumes of 0.85% NaCl, are far less infectious than equal numbers of organisms not separated from the medium in which they have grown.

The virulence of washed organisms is increased at least 10,000 fold by the simultaneous injection of nonlethal amounts of neutralized culture filtrate.

This aggressive activity of the culture filtrate is destroyed by heating to 70 C. for 30 minutes, and by the addition of the specific Welch antitoxin. It must be concluded from this that the aggressin of the filtrate and the toxin are identical.

This conclusion is strengthened by the fact that sublethal amounts of toxin made by the muscle culture method show a similar aggressive effect, which is likewise neutralized by the addition of antitoxin.

Nonspecific culture filtrates from cholera and proteus cultures do not increase the virulence of washed bacilli.

The aggressive substance (toxin) seems to act by reason of its necrotic effect and not by a negative chemotactic influence on leukocytes.

These experiments throw light on the mechanism of experimental infection and on the method of extension of that in the human body.